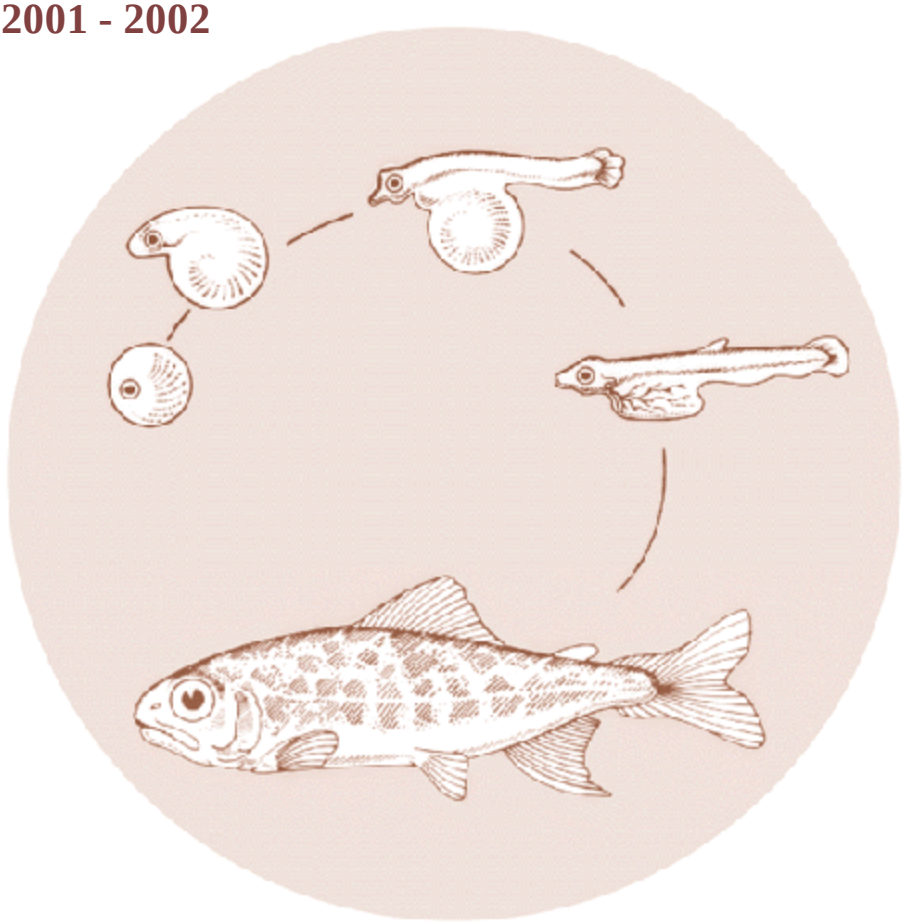
Redfish Lake Sockeye Salmon Captive Broodstock Rearing and Research

Annual Report
2001 - 2002



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REDFISH LAKE SOCKEYE SALMON CAPTIVE BROODSTOCK REARING AND RESEARCH, 2002

Annual Report

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EXECUTIVE SUMMARY

The National Marine Fisheries Service (NMFS) Northwest Fisheries Science Center, in cooperation with the Idaho Department of Fish and Game and the Bonneville Power Administration, has established captive broodstock programs to aid recovery of Snake River sockeye salmon (*Oncorhynchus nerka*) listed as endangered under the U.S. Endangered Species Act (ESA). Captive broodstock and captive rearing programs are a form of artificial propagation that are emerging as an important component of restoration efforts for ESA-listed salmon populations that are at critically low numbers. Captive broodstocks, reared in captivity for the entire life cycle, couple the salmon's high fecundity with potentially high survival in protective culture to produce large numbers of juveniles in a single generation for supplementation of natural populations.

The captive broodstocks discussed in this report were intended to protect the last known remnants of sockeye salmon that return to Redfish Lake in the Sawtooth Basin of Idaho at the headwaters of the Salmon River. This report addresses NMFS activities from 1 September 2001 to 31 August 2002 on the Redfish Lake sockeye salmon captive broodstock and captive rearing program. NMFS currently has broodstocks in culture from year classes 1997, 1998, 1999, 2000, and 2001 in both the captive breeding and captive rearing programs. Offspring from these programs are being returned to Idaho to aid recovery efforts for the species.

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INTRODUCTION

In 1992, the National Marine Fisheries Service (NMFS) Northwest Fisheries Science Center (NWFSC), in cooperation with the Bonneville Power Administration (BPA) and the Idaho Department of Fish and Game (IDFG) began a captive broodstock program for Redfish Lake sockeye salmon. These fish were listed as endangered under the U.S. Endangered Species Act (ESA) in 1991. From 1991 to 1998, a total of 16 wild adult anadromous sockeye salmon returned to Redfish Lake in the Stanley Basin of Idaho (Figure 1). Releases from the NMFS and IDFG captive broodstock programs generated seven returning adults in 1999, 257 returning adults in 2000 (Kline and Willard, 2001) and 26 returning adults in 2001. As of 31 August 2002, 23 adults had returned to the Stanley Basin for the 2002 season.

The captive broodstock program initially served as a gene conservation program to prevent the loss of this evolutionary significant unit. However, the high fecundity of Pacific salmon, coupled with increased survival in protective culture, allowed the program to produce large numbers of spawnable fish in a single generation. These fish provided a means of rapidly increasing the abundance of offspring available for restoration releases in the Stanley Basin lakes (Flagg et al. 1995, Schiewe et al. 1997). At every opportunity, the captive broodstock program incorporated adults returning from the sea to help ensure the continued adaptation of these fish to their natural environment.

The NMFS captive broodstocks continue to complement those reared by IDFG to reduce the risk of catastrophic loss from mechanical failure, human error, or disease. Prespawning adults, eyed eggs and juveniles from NMFS captive broodstocks are

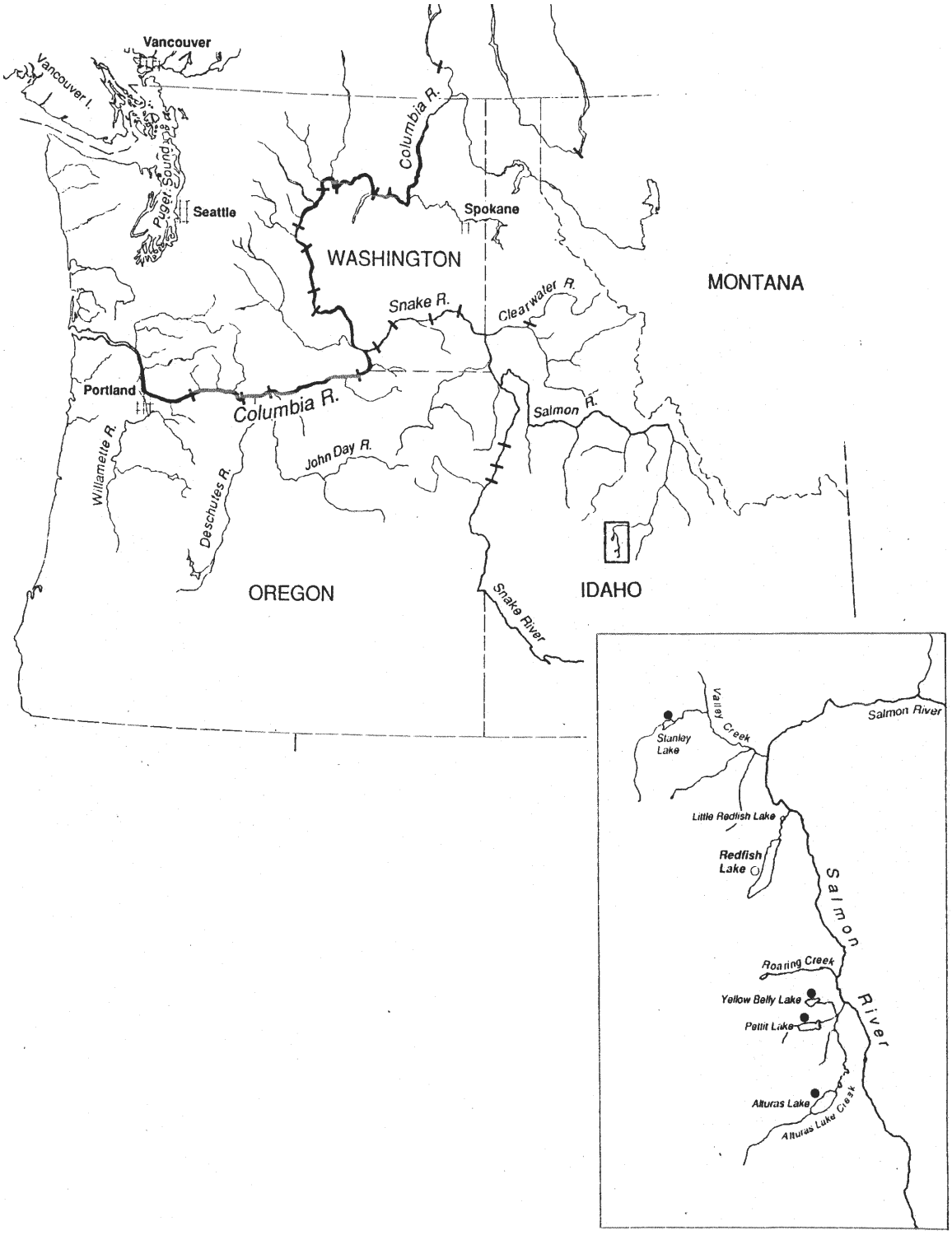

Figure 1. Map showing location of Redfish Lake. Sockeye salmon returning to Redfish Lake travel a greater distance from the sea (almost 1,450 km) and spawn at a higher elevation (almost 2,000 m) than any other sockeye salmon population.

provided to IDFG to assist in the recovery of self-sustaining runs of sockeye salmon in the Stanley Basin. Recovery activities for NMFS and IDFG Redfish Lake programs are coordinated by the Stanley Basin Sockeye Salmon Technical Oversight Committee (SBSTOC). Membership on the SBSTOC includes IDFG, NMFS, BPA, the Shoshone-Bannock Tribe, the University of Idaho, and private groups concerned with sockeye salmon restoration in Idaho. NWFSC staff participate in bimonthly SBSTOC meetings.

The following report summarizes the operation of the NMFS Redfish Lake sockeye salmon captive broodstock program from 1 September 2001 to 31 August 2002. The report describes the facilities, fish culture practices, and the status of the various rearing groups in the captive broodstock program.

FACILITIES

The NMFS Redfish Lake sockeye salmon captive broodstock project was divided between two facilities. The seawater facility is located at the NMFS Manchester Research Station (MRS) located on Clam Bay, a small bay adjoining the central basin of Puget Sound in Washington State. The MRS consists of several buildings with offices, laboratories, and a land-based seawater captive broodstock rearing complex. The freshwater phase of sockeye salmon rearing is conducted at Burley Creek Hatchery (BCH) near Port Orchard, Washington. A complete description of NMFS facilities can be found in Frost et al. (2002).

In cooperation with Oregon Department of Fish and Wildlife (ODFW), Redfish Lake sockeye salmon eggs were transported to and reared to the smolt stage at Bonneville Fish Hatchery (BFH) near Cascade Locks, Oregon for the smolt release program.

Facilities at BFH consisted of 10ºC well-water supplied vertical incubation trays, Canadian troughs (0.8-m by 0.5-m by 4.5-m), and 2.7-m diameter circular tanks in an enclosed building. In addition, fish were reared in outdoor raceways (6.1 m by 0.8 m by 24 m) supplied with either well water or 4 - 13ºC Tanner Creek water. ODFW provided personnel for daily facility activities.

REINTRODUCTION STRATEGIES

The NMFS captive broodstock program has successfully generated sockeye salmon for release into the Stanley Basin lakes (e.g., Redfish, Alturas, and Pettit) at several different life history stages to help restore ESA-listed endangered Snake River sockeye salmon. NMFS obtained appropriate permits for interstate transport of all fish and progeny.

1. Egg box releases—Eyed eggs from captive broodstock fish that were spawned at the NMFS freshwater facility were returned to Idaho for stocking egg boxes for in-lake hatching.
2. Presmolt releases—Eyed eggs from captive broodstock fish that were spawned at the NMFS freshwater facility were returned to Idaho for rearing and release during the presmolt stage.
3. Smolt release—Juveniles from captive broodstock program eggs sent to BFH were reared to smoltification and transported to Idaho for release.
4. Adult release—Prespawning (maturing) adults reared in captivity were returned to Idaho for release into Stanley Basin lakes for volitional spawning.

CAPTIVE BROODSTOCK FISH CULTURE PRACTICES

Each fall in 1998, 1999, 2000, and 2001 NMFS received eggs shipped from IDFG spawning of Redfish Lake sockeye salmon for the broodstocks discussed in the current reporting period. NMFS also received 310 smolts from IDFG in 1999 from its 1997 broodyear production group, and NMFS also retained a safety net of eggs from its spawnings in 1998, 2000, and 2001 to be used in either the captive broodstock or the adult release programs. In winter 2000, NMFS received a transfer of 1999-brood fry from IDFG for the adult release program.

The captive broodstock and adult release program fish were reared using standard fish culture practices and approved therapeutics (for a general overview of methods see Leitritz and Lewis 1976, Piper et al. 1982, FRED 1983, McDaniel et al. 1994, Schreck et al. 1995, Pennell and Barton 1996). Fish culture practices conformed to the husbandry requirements detailed in ESA Section 10 Propagation Permit 1148 for NMFS rearing of Idaho stocks of ESA-listed Snake River sockeye salmon. Generally, juvenile-to-adult rearing density in the tanks was maintained at less than 8 kg/m^3 (0.5 lb/ft^3) during most of the culture period; however, fish density ranged to 15 kg/m^3 (1.0 lb/ft^3) at maturity. Loading densities in freshwater ranged from 0.24 kg/Lpm (2.0 lb/gpm) to 0.84 kg/Lpm (7 lb/gpm). Seawater loading densities ranged up to 1.08 kg/Lpm (9 lb/gpm).

Individual genetic lots of eggs were placed in separate isolation containers and incubated to swim-up stage. The containers were periodically checked for dead eggs and/or alevins. At swim-up stage, the alevins were moved in their containers from the incubation room to blue plastic grow-out tanks (1.8-m diameter). The containers were placed into floating rings that held the container suspended in the tank water. The fry

remained in the containers until they reached approximately 0.7 g, at which time they were released into the tank. The fish were grown in these tanks until they were large enough to be PIT tagged (usually > 120 mm in length) and combined into larger rearing pools (3.7-m diameter). Fish maintained in the captive broodstock program were tagged with passive integrated transponder (PIT) tags as presmolts (Prentice et al. 1990) using a separate, disinfected needle for each fish. The individual tag codes were used to maintain individual fish pedigrees, which were utilized at spawning to determine mating crosses. Fish in the adult release program were not PIT tagged because individual genetic identity was inconsequential for rearing these volitionally spawning fish.

All large tanks (3.7 m-diameter and larger) used for sockeye salmon captive broodstock rearing were completely covered with a taut 2.5 x 2.5 cm or smaller mesh nylon netting to prevent fish from jumping out. In addition to the mesh, half of each tank was covered with black fabric to provide a covered refuge area fish could move under when disturbed.

A mild current (< 35 cm/sec) was generated in the rearing tanks by their circular shape, center drain, and a subsurface water jet inlet. This current provided a self-cleaning action in the tank and a very slight exercise potential. At least twice a week, bottom material that was not swept out of the tank by the current was removed by flushing.

Fish were reared on a commercial (e.g., Biodiet[1]) diet. Swim-up fry were fed a semi-moist starter "crumble". At about 1 g body weight, the fish were transitioned to a standard pelleted semi-moist grower ration and progressed through the "brood" rations as they grew. Daily ration ranged from 5.6% down to 0.4% body weight per day depending

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

on estimated fish size and water temperature (Iwama 1996). The ration was designed to grow the fish on the profile described in Figure 2, which was based upon periodic sample-weights of past broodyears. Pellet size was determined by the feed manufacturer's recommendations, based on current guidelines for commercial aquaculture and the guidance provided in Fowler (1989). When necessary, pellet size was adjusted from the chart recommendation to ensure the smallest fish in the population were able to feed.

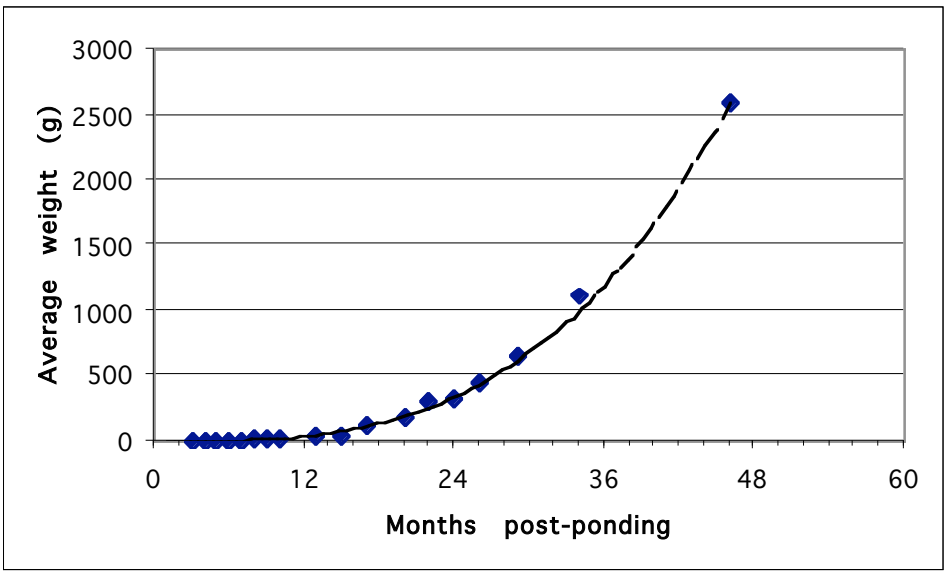

Figure 2. Growth rate projection for Redfish Lake sockeye salmon reared in 10°C freshwater at Burley Creek Hatchery. Growth profile based on historical data for sockeye reared in freshwater by NMFS.

Swim-up fry were started with hand feeding in 1.8-m diameter circular tanks. When the fish were greater than 30 g, they were PIT tagged and transferred to 3.7-m diameter circular tanks where their diet was either hand fed or rationed by automated feeders (Allen or belt feeders). Each day, prior to loading the feeders, a small portion of the day's ration was broadcast over the surface to observe the fish's feeding response.

The feeding frequency varied with day length, feeder type and fish size, as suggested by Fowler (1989).

In general, the fish were handled with extreme care and kept in water to the maximum extent possible during transport and processing procedures. Whenever possible, fish were transferred with a fish transfer tube that held water to prevent the added stress of a waterless transfer. Center standpipes in rearing tanks were constructed to hold at least 15 cm of water depth in the tank when the external standpipe was pulled to lower tank water level. This minimized the chance of fish being accidentally dewatered during tank draining or flushing. The fish were maintained on low levels of natural lighting and natural photoperiod. In order to determine adherence to the growth profile, the fish were subsampled occasionally to assess fish weight and length.

Transportation of juvenile or adult fish emphasized fish health and safety. All transportation occurred in insulated containers, and temperature was not allowed to rise more than 2°C. The transport containers were supplied with a continuous oxygen supply that maintained dissolved oxygen at full saturation. The oxygen reservoir contained at least twice the oxygen needed to make the entire trip. The containers were loaded at no more than 0.06 kg/L (0.5 lb/gallon).

Fish health was monitored in several ways. Fish were observed daily for feeding response, external condition, and behavior of fish in each tank as initial indicators of developing problems. In particular, signs of lethargy, spiral swimming, side swimming, flashing, unusual respiratory activity, body surface abnormalities, and unusual coloration were noted. A fish pathologist examined captive broodstock mortalities to determine cause of death. When a treatable pathogen was either detected or suspected, a NMFS

veterinarian, in consultation with IDFG fish health staff, prescribed appropriate prophylactic and therapeutic drugs. Medication was mixed with the feed, with dosage based on the fishes' weight. In addition, maturing fish were injected with erythromycin one month prior to spawning. Select mortalities were preserved as appropriate for pathology, genetic, and other analyses. After necropsy, specimens that were not vital to further analysis were disposed of in a manner consistent with ESA permits.

Redfish Lake sockeye salmon in the captive broodstock and adult release programs were reared to adulthood at NMFS facilities. During the year of expected maturation feeding was discontinued in late spring to coincide with expected time of Columbia River entry for wild fish. In the past, maturing fish could first be assessed during mid-summer when external signs of maturation became visible. Signs of maturation were determined by changes in skin sheen, skin coloration, and body morphology. This year, ultrasound examination in May and the beginning of July allowed earlier determination of gonad status to obtain estimates of sex ratios in the captive broodstocks. These data aided in separating maturing from nonmaturing fish to prevent nonmaturing fish from being taken off feed for an extended period of time. In late summer, fish in the adult release program will be transported from BCH to Idaho for release.

During the spawning season, typically after October 1, mature captive broodstock salmon were anesthetized with tricaine methanesulfonate (MS-222) and checked for ripeness on a weekly basis. Hormone implants consisting of gonadotropin releasing hormone analog (GnRHa) were injected into the dorsal musculature of some unripe fish, generally males with no milt output and females that felt firmer than others, to expedite

ovulation and spermiation to coordinate spawning timing between males and females (Swanson 1995). Fish that were ready to spawn, as determined by egg or milt expression (FRED 1983), were humanely killed and had their PIT tag, length, and weight recorded. Females were bled by severing the caudal vein. Bleeding reduces the amount of clotted blood in the eggs that might reduce fertilization by inhibiting sperm motility and penetration. Females were bled for 3 - 5 minutes, then abdominally incised and the eggs collected into a plastic bag. The eggs from each female were divided into two approximately equal lots. The bags of eggs were placed in a cooler with a small amount of ice to chill them until fertilization. Males were live-spawned, and milt was collected into Whirl-pak bags by compressing the ventral surface. Milt motility was checked visually with a microscope.

All spawned fish were analyzed for common bacterial and viral pathogens, e.g., *Renibacterium salmoninarum*, the causative agent for bacterial kidney disease (BKD), infectious hematopoietic necrosis virus, etc. Tissue samples collected from the kidney, spleen and pyloric caeca of each fish and ovarian fluid samples collected from each female were sent to a NWFSC lab for analysis. Egg transfers were based upon IDFG-established criteria for BKD enzyme-linked immunosorbent assay (ELISA) optical density (OD) levels. Only offspring from low ELISA females (OD < 0.2) were returned to Idaho. Eggs from females with moderate OD levels were sent to BFH for rearing to smoltification. Subsequent fish health sampling at BFH following the above criteria was used to determine whether the fish could be certified for return to Idaho as smolts. All eggs from females with high ELISA levels were culled to prevent the spread of BKD.

Mating strategies were structured to maintain genetic diversity. These strategies included pairing in as many different combinations as possible, avoidance of pairing between siblings, fertilization between different year classes and fertilization with cryopreserved sperm from other generations as suggested by Hard et al. (1992). A small tissue sample was taken from the caudal fin at spawning for DNA comparisons with outmigrating smolts and returning adults of Stanley Basin lakes.

Eggs were fertilized following "dry method" procedures. Milt from one male was pipetted into a plastic bag containing about half the eggs of one female. The eggs and milt were gently mixed for several seconds, well water was then added to activate the sperm, and the eggs were lightly agitated to distribute the activated milt. The bags were left undisturbed during the initial stages of the fertilization process. After about five minutes, most of the water was drained off, and the eggs were water hardened in a 1:100 buffered iodophore solution for 20 minutes and placed in white, 2-l plastic down-flow containers for isolated incubation. Beginning two days after fertilization, the eggs were treated with formalin injected into the water supply at 1,668 ppm for 15 minutes on alternating days for control of *Saprolegnia* spp. The eggs were left undisturbed during the sensitive period beginning 48 hours after fertilization until they reached the eyed stage. Eyed eggs were shocked and weighed. Dead or unfertilized eggs were removed and counted or weighed to determine eyed-egg viability rates.

Eggs to be shipped to Idaho or BFH were placed into open mesh plastic tubes (27-cm long by 6-cm diameter) at approximately 2,700 eggs per tube for IDFG and 2,000 eggs per tube for BFH. Each packed tube was wrapped in wet cheesecloth and placed in a small shipping container. Ice was placed in a top layer of cheesecloth to keep the eggs

cool and moist during shipment. Shipment to Boise, Idaho was by a common carrier flight of two hours. Shipment to BFH was 3.5 hours by motor vehicle.

SMOLT RELEASE FISH CULTURE PRACTICES

NMFS coordinated the Mitchell Act funded rearing of fish at BFH through the SBSTOC. For a description of the BFH Redfish Lake sockeye salmon rearing practices, please refer to Frost et al. 2002. NMFS coordinated the rearing and release of the smolts and obtained appropriate health certifications and permits for interstate transport of the smolts. NMFS coordinated all its transfers of eyed eggs and fish with the other agencies and tribes involved in Snake River sockeye salmon recovery through the SBSTOC. The release of these captive broodstock groups into the Upper Snake River Basin is expected to help restore a viable run of anadromous sockeye salmon to the area.

Unfortunately, an infectious hematopoietic necrosis virus (IHNV) outbreak occurred in May 2002 in the brood-year 2000 presmolts at BFH. Exposure to IHNV probably occurred during their brief residence in Tanner Creek water, when the wells were undergoing maintenance. The infection resulted in the death of more than 27,000 fish. The remaining brood-year 2000 fish were destroyed to reduce the chance that returning adults might carry the disease to Redfish Lake. A search is being conducted for an alternate smolt rearing facility.

SPAWNING AND REARING ACTIVITIES

During the reporting period, NMFS reared the following six Redfish Lake sockeye salmon genetic lineages in its captive broodstock program:

1. third generation progeny (brood-year 1998) of the one female and three male sockeye salmon that returned to Redfish Lake in 1991;
2. second (brood-years 1998 and 1999) and third generation progeny (brood-year 2000) of the one female sockeye salmon that returned to Redfish Lake in 1996;
3. second (brood-year 1997) and third (brood-year 2000) generation progeny of the one female that returned to Redfish Lake in 1994;
4. first generation progeny (brood-year 1999) of the single female and three of the six male sockeye salmon that returned to Redfish Lake in 1999;
5. first generation progeny (brood-year 2000) of females that returned to Stanley Basin lakes in 2000.
6. first generation progeny (brood-year 2001) of females that returned to Stanley Basin lakes in 2001.

Adult release fish transfer

This adult release group was transferred from IDFG brood-year 1997 production group at Sawtooth Hatchery to MRS in May 1999 as smolts for seawater rearing. Survival from smolt to age-4 adult was 64%. In September 2001, the fish (N = 141) were sorted and 68 maturing fish were transported to Stanley Basin lakes for volitional spawning under ESA Permit 1148, WDFW Permit 01-8-3513, ODFW permit letter dated 20-August-2001, and IDFG Permit HQ-01-067. Because they were not fully mature,

exact determination of sex ratios was not possible. However, it was estimated that there were at least 34 males and 47 females, with five fish of undetermined sex. These fish averaged 56 cm and 2.5 kg in size. Eighteen maturing fish were retained for spawning with the captive broodstocks (described below), and 55 immature fish were retained for continued rearing.

Spawning

In fall 2001, spawning began October 7 and continued through November 15 (Table 1). Survival to eyed-egg stage was inexplicably low for most spawn days. It was unknown whether the eggs were unfertilized or fertilized but ceased development some time during incubation. Broodstocks spawned included 1997-brood adult release, 1998-brood captive broodstocks, 1998-brood adult release, and 1999-brood captive broodstock. Spawning protocols followed those described earlier in this report. Because IDFG projected enough eyed eggs from their program to fulfill Stanley Basin lakes egg and presmolt outplanting goals, all viable eyed eggs from NMFS spawnings were transported to BFH for the smolt release program.

Adult release fish spawning

Eighteen maturing 1997 brood-year adult release program fish (nine females and nine males) reared in filtered seawater and transferred to freshwater for final maturation were retained at BCH to be included in NMFS fall 2001 spawning in October for additional genetic diversity in the captive broodstock spawning matrix. The females averaged 58 cm and 2.5 kg. With an average fecundity of 2,827 and an average egg viability of 47.1%, these fish produced 11,979 eyed eggs (Table 2). Unfortunately, four females had high BKD ELISA OD levels, resulting in their eggs (N = 5,959) being

culled. A total of 5,995 eyed eggs from healthy females were sent to BFH for the smolt release program. An additional 25 eggs were retained for NMFS brood-year 2001 adult release program. The nine mature male fish averaged 59 cm and 2.8 kg. Milt from these males was used to fertilize eggs from 1997 brood-year adult release females, 1998 brood-year adult release females that had been retained because of incidence of BKD in their population, and 1998 brood-year captive broodstock females.

The adult release brood-year 1998 group was comprised of fish from the 1998 spawning of an age-2 1996 brood-year female crossed with an age-2 1996 male. This half-sib group was reared at MRS. During their seawater rearing these fish incurred outbreaks of BKD and *Flexibacter maritimus* and were treated with Erythromycin. Because of these health issues these fish were not returned to Idaho, but remained with NMFS and were spawned at BCH in fall 2001. In August 2001, these fish were assayed for sex and maturity using ultrasound. A total of 52 maturing females and five maturing males from the 1998 broodyear adult release program were transported to BCH for final maturation. Survival from smolt to adult was about 67%. The males averaged 56.4 cm and 2.6 kg in size. The females' size ranged from 32.9 to 54 cm and 0.4 to 2.2 kg, with an average of 46.6 cm and 1.3 kg. With an average fecundity of 1,949 eggs and an average eyed-egg viability of 42.4%, a total of 41,294 eggs were produced (Table 2). Unfortunately, 11 females had high BKD ELISA OD levels, resulting in their eggs (N = 6,889) being culled. A total of 34,180 eyed eggs from healthy females were sent to BFH for the smolt release program. An additional 225 eggs were retained for the NMFS brood-year 2001 adult release program.

Captive broodstock spawning

The 1998 captive broodstock was comprised of two distinct half-sib groups sharing a common father. The first group of fish was the second-generation (F2) progeny from IDFG 1996 captive broodstock from the lone returning female sockeye salmon to Redfish Lake in 1996. The eggs from this broodstock were fertilized with milt from the lone returning wild male in 1998. IDFG transferred 55 eggs to NMFS in 1998. The second group contained third-generation (F3) progeny (descendents of the IDFG 1991-brood wild fish) from the 1998 spawning of NMFS 1994 brood-year sockeye that had been reared to smoltification at BFH and then transferred to MRS for rearing to maturation. These eggs were also fertilized with milt from the lone returning male sockeye salmon to Redfish Lake in 1998. These fish were PIT tagged and combined into common rearing tanks in 1999, remaining in freshwater through adulthood. In September 2001, the maturing brood-year 1998 Redfish Lake sockeye salmon were injected with erythromycin as a prophylactic treatment prior to spawning. GnRHa implanting of males took place throughout the spawning period as fish were sorted for ripeness. Females that seemed to be ripening slowly were implanted with GnRHa later in October. A total of 43 females were spawned, but five females were only partially ovulated when spawned. The females averaged 50.8 cm and 1.8 kg. A total of 38 males matured, which averaged 52.8 cm and 2.0 kg. Of the total number of fish that matured, ten females and six males were from the F2 generation and 33 females and 32 males were from the F3 generation. Survival of the F2 generation from ponding to age three adults was 68%. Survival for the F3 generation was about 64%. The F2 generation fecundity averaged 2,082 (range 881 – 2,743; 1,301 eggs/kg of female weight) (Table 2). The average egg viability was 61.7%.

The F3 generation fecundity averaged 2,339 (range 1,140 – 3,412; 1,299 eggs/kg of female weight). The average egg viability was 58.4%. All parents met IDFG BKD ELISA criteria, resulting in no eggs being culled for this 1998 broodyear of sockeye salmon. All eggs produced during the 2001 spawning season, with the exception of those retained for the adult release program, were transported to BFH for the smolt release program. Approximately 11,440 eyed eggs from F2 generation parents and 38,115 eyed eggs from F3 generation parents were transported to BFH for the smolt release program in November and December 2001 under ESA Permit 1148, WDFW Transfer Permit 01-11-3573 and ODFW Authorization Letter issued 14 November 2001. In addition, the 2001 brood-year adult release program was stocked with 125 eggs from each of the F2 and F3 generation 1998 brood-year lineages.

NMFS is currently maintaining 1999 brood-year captive broodstock and adult release groups at BCH. The captive broodstock group is comprised of second generation fish from IDFG's 1996 brood-year captive broodstock and first generation fish from the lone returning female sockeye salmon to Redfish Lake in 1999. In fall 2001, an unexpectedly large number of fish matured at age-2, probably due to accelerated feeding early in their development (ad lib during early fry stage). A total of 185 fish (51% of the population) matured, resulting in 178 males and 7 females. Males averaged 34.7 cm and 0.5 kg in size. Females averaged 34.1 cm and 0.5 kg in size. About 1,587 green eggs were obtained from two of the females, but only one female's eggs were viable. The productive female produced 713 green eggs. An eyed-egg viability of 88.4% resulted in about 630 eyed eggs. These eggs were sent to BFH for the smolt release program. The overall eyed-egg viability of the two 1999 brood-year females that were spawned was

39.7% (Table 2). The remaining females were either unripe when spawned or were not separated from the males during maturity sorting due to the difficulty in discerning sexes in the age-2 fish that were not fully mature. The milt from 16 males was used to fertilize eggs from 1998 brood-year captive broodstock females.

Fish Culture

Brood-year 1997

This adult release group was transferred from IDFG's brood-year 1997 production group to MRS in May 1999 as smolts for rearing to maturity in seawater. In September 2001, the majority of these fish (n = 68 out of 141) matured and were transported to Stanley Basin lakes for volitional spawning. Eighteen mature fish were retained at BCH for spawning. Unexpectedly, the remaining 55 fish did not mature in 2001 and remained at BCH until they succumbed to BKD by June 2002. Overall survival from smolt stage was 47% (Table 3). About 90% of the immature age-4 fish were males.

Brood-year 1998

Captive broodstocks--The 1998 captive broodstock was comprised of two distinct half-sib groups (F2 generation and F3 generation) sharing a common father, as described in a previous section. NMFS retained 400 eggs, 254 of which survived to hatch. NMFS transferred 127 of the surviving eggs to IDFG and retained 127 for captive broodstock. After PIT tagging in 1999, these two half-sib groups were combined. Survival for the F2 generation fish from ponding to August 31 has been 63% (45% from eyed egg) (Table 3). Survival for the F3 generation fish has been 63% from ponding (61% from eyed egg). The remaining 14 fish are expected to spawn in 2002 at age 4.

Adult release--The second brood-year 1998 group was comprised of fish from the 1996 brood-year half-sib group that was dedicated to the adult release program.

Because of BKD and *Flexibacter maritimus* health issues, these fish were not returned to Idaho, but remained with NMFS and were spawned at BCH in fall 2001. Ten fish that did not mature remained in seawater at MRS. Unfortunately, these fish succumbed to BKD by June 2002. Survival from eyed egg to maturation for this group was 65% with almost all the mortality (32.5 of the 35.0%) occurring after the fish were transferred to seawater as smolts (Table 3).

Brood-year 1999

Captive broodstocks--NMFS is currently maintaining a captive broodstock and an adult release group of 1999 brood-year fish at BCH. The captive broodstock group is comprised of second generation fish from the IDFG 1996 brood-year captive broodstock and first generation fish from the lone returning female sockeye salmon to Redfish Lake in 1999. NMFS currently has 169 of these fish in culture. Survival from the eyed egg stage has been 61% through August 2002 (Table 3). Most mortalities occurred during the first two months after ponding.

Ultrasound scanning in July determined that all but three of these fish were maturing as 3-year olds and that approximately 40% of the fish in the population were females. These fish will be spawned in fall 2002, and the resulting eyed eggs returned to Idaho for recovery efforts.

In the summer of 2002 these fish were utilized in an exploratory investigation of the effect of exercise on milt production. Captive broodstock fish are usually held in circular tanks with current velocities that are less than they would encounter during natural upstream migration. Theoretically, the exercise generated during upstream migration may generate physiological conditions that promote the proper development of

reproductive characteristics. Redfish Lake sockeye salmon captive broodstock have experienced low volume milt production as well as delayed ovulation and even more delayed sperm production that maybe responsible for reduced eyed egg viability in program fish. To test whether exercise or exertion plays a role in male and female spawn timing and milt volume in males, two raceways were each stocked with 10 females and 20 males. Both raceways had water inflow at one end supplying fresh water. One raceway was equipped with an auxiliary recirculating pump that cycled water through jets spaced throughout the raceway above the water and directed at the surface of the water at an angle to create a current. This preliminary test was intended to start in early summer to coincide with river entry of wild fish. However, due to scheduling constraints, the test did not begin until August, near the end of this reporting period. As this was a preliminary test to determine the feasibility of conducting this type of evaluation, and because of the late start, no significant results are expected. This test is expected to be repeated next year with improved scheduling and replication.

Adult Release--The adult release group contains first generation offspring from the lone-returning program female sockeye salmon in 1999 and second generation offspring from the IDFG brood-year 1996 captive broodstock. Survival through August 2002 has been about 45%, with the primary cause of mortality being accidental exposure to iodophore in a disinfected tank into which the fish were moved in 2000 (Frost et al. 2001). Approximately 131 fish will be transported to Idaho for release in September 2002 (Table 3).

Brood-year 2000

Captive Broodstocks--In fall 2000, NMFS received 347 eggs from IDFG for the captive broodstock program. These fish are the result of matings between males and females from several different lineages, including anadromous-returning sockeye salmon (1993- and 1996-broods) and captive-reared sockeye salmon (1997- and 1998-broods) from IDFG Eagle Hatchery. In addition, IDFG transported an additional 42 fry in February 2001. Egg to ponding survival was 98%. Four fish were culled as early fry in 2001 due to "pinheadedness" or a failure to thrive, and one was culled due to a spinal deformity. NMFS currently has 348 of these fish in culture (Table 3). Survival in the individual groups ranges from 80 to 100%, with an overall survival of 92% from ponding, and 90% from eyed egg. In May 2002 these fish received a newly developed trial vaccine for BKD.

Fish in this broodyear are involved in an evaluation of the effects of growth rate modulation on age at maturation. In recent years, unexpectedly high rates of precocious male maturation have occurred within the NMFS captive broodstock population. Recent analysis of age-at-maturity data suggests that size at the first May after ponding may determine, at least in part, the maturation schedule. The BY 2000 sockeye salmon were fed a ration so that growth rate approximated the trajectory of BY 1993 sockeye salmon which had a low (2%) rate of male precocity. After PIT tagging in December 2001, the fish were divided into four rearing groups. Two groups were fed an accelerated ration and the two remaining groups continued on the standard ration. Monthly size-sampling was conducted to track growth. The final sampling and maturation determination will be conducted in September 2002 to determine whether growth rate affected age-2

maturation. Preliminary observations of these fish during the August size sampling have failed to detect any early maturing fish. However, the August sampling was a partial sample, with 25% of the fish individually examined. Still, the maturation rate is expected to be low (less than 1%) based on visual observation of fish in the tanks. Final sampling in September will determine the final maturation rate.

Adult release--NMFS retained 538 eggs from the 1997 F2 captive broodstock females (from the single 1994 anadromous female) for the adult release program. Egg to ponding survival was 83% for these fish. In May 2002, approximately 200 fish in this group were transferred to MRS for seawater rearing. The current overall survival for this adult release group is about 72% with 389 fish remaining in culture (Table 3). It is anticipated maturing fish from this group will be returned to Stanley Basin lakes for volitional spawning in the Fall of 2003 and 2004.

Smolt release--In fall 2000, NMFS transported 37,900 eggs from healthy brood-year 1997 F2 parents (ELISA OD < 0.2) to BFH for the smolt release program. These eggs were combined with about 1,300 eggs from brood-year 1996 F2 females from NMFS, plus 70,149 eggs from IDFG captive rearing program for a total of 109,300 eggs for the smolt release program. ODFW ponded 96,096 alevins in February 2001. These fish were coded-wire tagged in fall 2001 and 2,000 fish were PIT tagged in February 2002 for release in May 2002.

In May, infectious hematopoietic necrosis virus (IHNV) was detected in the 2000 broodyear Redfish Lake sockeye salmon smolts at BFH approximately two weeks before their transport and subsequent release to Idaho. Further testing and mortalities confirmed the initial diagnosis. On the basis of this, it was determined that the fish could not be

returned to Idaho, where IHNV has not been detected in Redfish Lake sockeye salmon populations. In June, approximately 40,596 smolts were destroyed to prevent the spread of this disease.

Brood-year 2001

Captive broodstocks--In fall 2001, NMFS received a total of 435 eggs from IDFG spawning of brood-year 1998 F2 females and of program females returning to Stanley Basin lakes. These eggs were transferred to NMFS facilities under ESA Permit 1120 and WDFW Permit 01-11-3574 where they were placed into the captive broodstock program. Survival of these fish from eyed egg through August 2002 has been 79.5% (Table 3). Most mortalities occurred during the first month after ponding when Siamese twins and alevins with scoliosis were culled from the population. It is expected fish in this year class will mature in the fall of 2004 and 2005. These fish were placed in the same growth modulation evaluation conducted last year with brood-year 2000 fish. Their growth rate has been maintained similar to the 2000 brood-year fish resulting in an average weight of 2.7g during their first May after ponding. In the Fall of 2002 these fish will be PIT tagged and divided into two groups to replicate last year's experiment.

Adult release--In fall 2001, NMFS retained 500 eggs from brood-year 1997 and 1998 females for the brood-year 2001 adult release program. Survival of these fish to August 2002 has been 80% (Table 3) with most mortality occurring in the first 3 months post ponding from unknown causes. The current plan is to return maturing fish in 2004 and 2005 to Stanley Basin lakes for volitional spawning.

Smolt release--In fall 2001, NMFS sent a total of 90,222 eyed eggs to BFH for the smolt release program. Well failure at BFH precluded the possibility of rearing these

fish through the smolt stage on pathogen free water. Therefore, the decision was made to transfer these fish back to the Stanley Basin for early release. In August 2002 the fish were adipose fin and ventral fin clipped in preparation for release at the end of the month. In addition, 4,000 fish were PIT tagged to monitor overwinter and outmigration survival. Approximately 75,428 presmolts were transported and released into Redfish, Alturas and Pettit Lakes between 27-29 August 2002. Unfortunately, equipment failure resulted in the death of 2,195 fish en route to Pettit Lake. The average survival of these fish from egg to release was 83.6%. The fish averaged 11 g at release.

SUMMARY

The program has successfully maintained this ESA-listed population in captivity and provided fish for use in restoration. The captive broodstock program provides both freshwater and seawater rearing facilities that help ensure the retention of anadromous traits. Fish have been successfully reared for over a year at the new BCH freshwater rearing facility. This program, united with the IDFG captive broodstocks, should continue to provide for the restoration of self-sustaining natural runs of anadromous sockeye salmon to Stanley Basin lakes.

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TABLES

Table 1. Fecundity and egg viability for 1997 brood age-4, 1998 brood age-3, and 1999 brood age-2 Redfish Lake sockeye salmon spawned at NMFS facilities, 2001.

Date of spawning	Females spawned	Stocks[1]	Green eggs (n)	Eyed eggs (n)	Viability (% to eye)
Oct. 7	20	1, 2	42,195	25,131	59.6
Oct. 10	15	1, 2	30,673	19,148	62.4
Oct. 12	5	2, 3	9,148	3,137	34.3
Oct. 17	9	1, 2, 3, 4	20,917	8,372	40.0
Oct. 22	20	2, 3, 4	37,079	4,305	11.6
Oct. 25	5	3, 4	10,338	7,070	68.4
Nov. 2	18	3, 4	39,005	20,505	52.6
Nov. 5	3	4, 5	6,159	4,471	72.6
Nov. 9	1	4	2,889	2,799	96.9
Nov. 15	4	4, 5	10,283	8,769	85.3
Totals	100		208,686	103,707	49.7

[1] 1 = BY 97 Adult release group reared to maturity in filtered seawater
2 = BY 98 Adult release group reared to maturity in filtered seawater
3 = BY 98 F2 Captive broodstock reared to maturity in freshwater
4 = BY 98 F3 Captive broodstock reared to maturity in freshwater
5 = BY 99 Captive broodstock reared to maturity in freshwater